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Resurrection plants are unique in that the vegetative tissues have the ability to dry to 5% relative water content (RWC) and recover full metabolism in existing tissues on rehydration. The mechanisms whereby this is achieved varies among the orders. In bryophytes, where drying and rehydration is rapid, tissues suffer damage during drying but is repaired upon rehydration. In the angiosperms, extensive protection is laid down during protracted drying and little by way of repair is required. The pteridophytes include several resurrection species, but it is not known whether survival is by protection, repair or a combination of both. The present study was undertaken to determine the mechanism of tolerance in the resurrection fern *Morhia caffrorum*. Plants were collected from Table Mountain in summer (the dry season) and maintained in a glass house before and during experimentation. Morphological (frond folding), anatomical and ultrastructural (SEM and TEM), physiological (relative water content [RWC] and electrolyte leakage) and biochemical (quantification of sugars, LEAs and antioxidants) were assessed during drying and rehydration of plants. Plants dried in summer were desiccation tolerant. Fronds dried to 5% RWC had minimal electrolyte leakage and recovered full turgor on rehydration. They curled inwards and chlorophyll shading occurred facilitated by a dense layer of adaxial scales. Sucrose levels increased and a number of heat stable LEA-like proteins were produced *de novo* during drying. These declined during rehydration to levels present in pre-dried fronds. The enzymic antioxidants, ascorbate peroxidase, catalase, glutathione reductase and superoxide dismutase, remained active during the desiccation and rehydration. Subcellular organisation was retained without evidence of damage. During the winter months, when rain is prevalent (albeit that they did not experience the rain), the plants lost the ability to recover from desiccation stress. Fronds did not curl during drying and full rehydration did not occur. Sucrose levels did not increase and no new heat stable proteins appeared upon dehydration. Antioxidant enzymes became denatured and lost activity upon dehydration. During winter, the plants produces spores which became desiccation tolerant and have all the same characteristics of desiccation tolerant "summer" fronds. Upon germination of these spores, an event which occurs in spring and early summer, the new ferns were once again desiccation tolerant. To our knowledge, this is the first report of a species in which desiccation tolerance is seasonal.

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Associations between the biophysics of water and desiccation stress in *Vitellaria paradoxa* seeds

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We previously showed for two batches (1996, 2002) of *Vitellaria paradoxa* (Sapotaceae) seeds that smaller seeds in the population dried most rapidly and that there was a significant linear relationship between whole-seed water content and seed mass during the drying process. Moreover, we showed that only the largest seeds in the population retained viability after drying to whole seed mean target moisture contents, i.e. larger seeds were not more desiccation tolerant but survived as a result of taking longer to desiccate. We then calculated the critical water content (CWC) for viability loss, assuming that the smallest seeds were killed first. Using this approach, we showed that surviving seeds were always above a single CWC, which was seed-lot-specific (c. 20% and 26% FWB in 1996 and 2002, respectively). Analysis of data for this species and 24 other tropical species revealed that a less steep slope for the desiccation mortality curve (% germination against % water content) was associated with increased seed-lot heterogeneity in mass. We concluded that the wide range of desiccation sensitivities typically inferred from such curves is an artefact of seed-to-seed variation in mass, and hence water contents, during drying. In this study we explore, in the same two seed lots of *Vitellaria paradoxa* previously investigated, whether critical water contents are related to: (1) specific biophysical events, such as turgor loss (based on isotherm analysis); (2) the status of water in the seed tissues (using differential scanning calorimetry); and (3) temporal and spatial variation of water across the seed during the drying process (using magnetic resonance imaging).

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Structural and metabolic changes associated with desiccation and rehydration of the roots of a dicot horticultural plant, *Ranunculus asiaticus*

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Ranunculus asiaticus is a horticultural dicot, the tuberous roots of which are able to tolerate desiccation (to 8–10% water content) during its annual life cycle. Root development prior to desiccation is accompanied by changes in the cell walls, with considerable thickening taking place. An increase in starch grains also occurs, and as the root undergoes desiccation there is also a substantial increase in protein. Upon rehydration of the roots, secondary walls expand rapidly upon water influx, presumably due to the extensive presence of pectin therein. As the aerial parts of the plant are formed from latent buds, and photosynthesis becomes established, there is depletion of the cell wall and cell contents of the roots, presumably to provide an early source of carbon and nitrogen. Recent studies have concentrated on the nature of the proteins that are present in the root during and following desiccation. There is a large increase in a particular low mol. mass protein of approx. 14 kDa, and this has been designated as a putative storage protein (JR14). Other stress-related proteins (dehydrins and smHSPs), which are often associated with tolerance of water deficits also increase. SDS-PAGE analysis of roots at all stages of the *R. asciaticus* life cycle revealed that JR14 accumulates during the desiccation period, and two-dimensional electrophoresis demonstrated that there are, in fact, two major proteins of around 14 kDa. One of these was purified, and the first 31 N-terminal amino acids were determined by sequencing. A 489 bp full length JR14 cDNA sequence was obtained by 3′-RACE and 5′-RACE PCR using oligonucleotide sequences based on the terminal amino acids. The amino acid sequence (163 amino acids) of the JR14 protein shows that it has stress-induced domains PLAT/LH2. A peptide of 24 N-terminal amino acids of the full length JR14 protein is a possible signal peptide, indicative that it is a sequestered protein. It has no strong similarity with known water-stress-related proteins, nor with the low mol. mass storage protein present in the tap roots of dandelion. Western blots indicated that the expression of dehydrin Dhn4B (LEAII D-11 family) is induced by dehydration and ABA, and declines during rehydration. The cDNA sequence of *Dhn4B* has a very high similarity with dehydrins known in other species. As with dehydrins, smHSPs were also detected by western blots in the tuberous roots during desiccation, and their expression was induced by high temperature and dehydration.

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The effect of developmental status on successful cryopreservation of recalcitrant seed germplasm

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The zygotic germplasm of plant species producing desiccation-sensitive seeds can be conserved in the long-term only by cryopreservation. Usually the embryonic axis is excised from the cotyledons and is used as the explant for cryopreservation, as it provides a favourable surface area:volume ratio. However the shoot of the axis of most species studied does not develop after excision, with the result that survival after cryopreservation is often recorded as callus production or simply greening. Thus, besides explant size, factors such as *in vitro* regeneration techniques, physical damage induced upon excision and developmental status of the seed could compromise the success of cryopreservation. This study investigated the effect of the factors mentioned above, with particular attention to the developmental status of the seeds, on *in vitro* development and cryopreservation of the desiccation-sensitive embryonic axes of three species: *Trichilia dregeana*, *T. emetica* and *Strychnos gerrardii*. For all three species, investigations were conducted on the embryonic axes excised from mature seeds immediately after harvesting and from mature seeds stored under hydrated conditions for different periods (in order to achieve different degrees of development). When germinated *in vitro*, excised embryonic axes of *T. dregeana* and *T. emetica* did not develop shoots unless the axes were excised with attached cotyledonary segments. Following the development associated with short-term storage, however, the axes could develop normally without any attached cotyledon. The embryos from the (endospermous) seeds of *S. gerrardii* developed normally without any attached cotyledon, but the percentage germination increased with seed storage period. For all three species, *in vitro* axis germination was optimal when activated charcoal was included in the germination medium, regardless of the developmental stage of the seeds. On dehydration to approximately 0.3 g g^{-1} (dry mass), embryonic axes from all three species failed to develop shoots even though more than 50% of explants produced roots. Hence, shoot production was more sensitive to desiccation than was root production. The sensitivity of shoots to desiccation did not change with seed storage for *T. dregeana* and *T. emetica*, but decreased for *S. gerrardii* when the seeds were stored for 6–8 weeks. Subsequent cryopreservation of *T. dregeana* and *S. gerrardii* explants was best achieved with rapid cooling in nitrogen slush (−210°). However, the optimal cooling procedure for successful cryopreservation of *T. emetica* explants is still to be established. The highest post-cryopreservation survival of *T. dregeana* axes was achieved when seeds had been stored for three months, while the seed storage period did not affect post-thaw survival of the axes of *T. emetica* and *S. gerrardii*. Only *S. gerrardii* explants produced shoots after cryopreservation, whereas the surviving embryonic axes of *T. dregeana* and *T. emetica* regenerated only as non-embryogenic callus. Thus, the successful cryopreservation of the germplasm of the species tested, and others producing recalcitrant seeds, depends on numerous species-specific factors.

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Seedling survival after desiccation when rehydrated with NaCl solutions, in some annual and perennial desert halophytes

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Seedling of the perennial desert halophytes *Haloxylon aphyllum*, *Salsola dendroides*, *Kochia scoparia* and *Kochia prostrata*, of the Chenopodiaceae family, from the Solonchak salinities of Kyzylkum desert in Uzbekistan were tested for their survival when germinated and rehydrated with NaCl solutions after periods of desiccation. The seeds were tested for germination in distilled water as well as in NaCl solutions of up to 3%, in light and darkness. The seeds germinated well in